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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF PREVENTION, PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

OPP OFFICIAL RECORD
HEALTH EFFECTS DIVISION
SCIENTIFIC DATA REVIEWS
EPA SERIES 361

November 13, 2007

TXR # 0054637

SUBJECT: MANCOZEB. Review of Acute Neurotoxicity Study (MRID 47126201).

PC Code: 014504
DP Barcode: D340145

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THROUGH: Michael Metzger, Branch Chief
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Conclusions: Attached is a review for an acute neurotoxicity study with mancozeb. The LOAEL is 500 mg/kg/day, the lowest dose tested, based on decreased motor activity on day 1 in males and females. The NOAEL was not determined. This study is classified acceptable/guideline and satisfies the guideline requirement for an acute neurotoxicity study in rats.

Results from this study will not change the endpoint for acute dietary exposure used in the most recent mancozeb risk assessment (D327307 & D327318, 6/11/07).

Although a NOAEL for motor activity was not determined in the acute neurotoxicity study, the lowest dose is believed to be close to a NOAEL because there was a lot of variability in the data. Applying an uncertainty factor of 3x to extrapolate a NOAEL would result in a dose of 167

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mg/kg/day which is greater than the NOAEL currently used to assess acute dietary risk (128 mg/kg/day) in the mancozeb risk assessment.

Executive Summary: In an acute neurotoxicity study (MRID 47126201), groups of fasted, 6-week old Fischer 344 rats (10/sex/dose) were given a single gavage dose of Mancozeb (83.8% a.i., Lot No. RK2888R232) in 0.5% aqueous Methocel™ at doses of 0, 500, 1000, or 2000 mg/kg (limit dose) and observed for 14 days. A functional observational battery (FOB) and motor activity testing were performed on all animals during pre-exposure, Day 1 (at 5 hours post-dosing, the time-of-peak effect), and Days 8 and 15. At study termination, 5 animals/sex/group were perfused *in situ* for neuropathological examination. The brain and peripheral nervous system tissues collected from the perfused animals in the control and 2000 mg/kg/day groups were subjected to histopathological evaluation. Positive control data were provided.

No compound-related effects on mortality, body weight, body weight gain, FOB, or gross pathology were observed at any dose in either sex. The only clinical sign noted was perineal fecal staining in several treated animals. On day 1 there was decreased total session motor activity in comparison to controls in all male groups (-25% to -31%) and all female treatment groups (-20% to -35%). Degeneration of an individual nerve fiber with myelin ovoid formation was seen in the proximal sciatic nerve of one male in the 2000 mg/kg group and in the tibial nerve of two males in this dose group. These lesions were similar to those seen in a subchronic neuropathology study with mancozeb and are attributed to treatment.

The LOAEL is 500 mg/kg/day, the lowest dose tested, based on decreased motor activity on day 1 in males and females. The NOAEL was not determined. This study is classified acceptable/guideline and satisfies the guideline requirement for an acute neurotoxicity study in rats (870.6200a; OECD 424).

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Template version 02/06

TXR#: 0054637

DATA EVALUATION RECORD

STUDY TYPE: Acute Neurotoxicity - Rats OPPTS 870.6200a; OECD 424.

PC CODE: 014504 **DP BARCODE:** D340145

TEST MATERIAL (PURITY): Mancozeb (83.8% a.i.)

SYNONYMS: ((1,2-Ethanediylbis(carbamodithioato))(2-)) manganese mixture with ((1,2-ethanediylbis(carbamodithioato))(2-)) zinc; Dithane M-45; GF-1042; RH-38165

CITATION: Maurissen, J.P., A.K. Andrus, and K.A. Johnson (2005) Mancozeb: Acute neurotoxicity study in F344/DuCrl rats. Toxicology and Environmental Research and Consulting, Dow Chemical Company, Midland, MI. Laboratory Study ID: 051080, November 7, 2005. MRID 47126201. Unpublished.

SPONSOR: Mancozeb Task Force, c/o McDermott, Will & Emery, LLP, 600 13th Street, NW, Washington, DC

EXECUTIVE SUMMARY: In an acute neurotoxicity study (MRID 47126201), groups of fasted, 6-week old Fischer 344 rats (10/sex/dose) were given a single gavage dose of Mancozeb (83.8% a.i., Lot No. RK2888R232) in 0.5% aqueous Methocel™ at doses of 0, 500, 1000, or 2000 mg/kg (limit dose) and observed for 14 days. A functional observational battery (FOB) and motor activity testing were performed on all animals during pre-exposure, Day 1 (at 5 hours post-dosing, the time-of-peak effect), and Days 8 and 15. At study termination, 5 animals/sex/group were perfused *in situ* for neuropathological examination. The brain and peripheral nervous system tissues collected from the perfused animals in the control and 2000 mg/kg/day groups were subjected to histopathological evaluation. Positive control data were provided.

No compound-related effects on mortality, body weight, body weight gain, FOB, or gross pathology were observed at any dose in either sex. The only clinical sign noted was perineal fecal staining in several treated animals. On day 1 there was decreased total session motor activity in comparison to controls in all male groups (-25% to -31%) and all female treatment groups (-20% to -35%). Degeneration of an individual nerve fiber with myelin ovoid formation was seen in the proximal sciatic nerve of one male in the 2000 mg/kg group and in the tibial

nerve of two males in this dose group. These lesions were similar to those seen in a subchronic neuropathology study with mancozeb and are attributed to treatment.

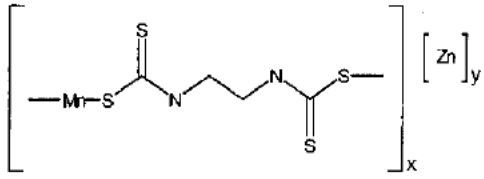
The LOAEL is 500 mg/kg/day, the lowest dose tested, based on decreased motor activity on day 1 in males and females. The NOAEL was not determined. This study is classified **acceptable/guideline** and satisfies the guideline requirement for an acute neurotoxicity study in rats (870.6200a; OECD 424).

COMPLIANCE: Signed and dated Data Confidentiality, GLP Compliance, Flagging, and Quality Assurance statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Mancozeb

Description:	Yellow powder
Lot/batch #:	RK2888R232
Purity:	The test material was determined to be 83.8% Mancozeb and 0.16% ethylenethiourea
Stability:	Shown to be stable in the vehicle for at least 24 hours at room temperature
CAS # of TGAI:	8018-01-7
Structure:	

2. Vehicle: 0.5% aqueous methylcellulose

3. Test animals

Species:	Rat
Strain:	Fischer 344/DuCrl
Age/mean weight at dosing:	6 weeks/ 81.7-86.5 g males; 79.9-81.4 g females
Source:	Charles River Laboratories (Raleigh, NC)
Housing:	Individually in suspended, stainless steel cages with wire mesh floors
Diet:	Pelleted LabDiet® Certified Rodent Diet #5002 (PMI Nutrition International, St. Louis MO), *ad libitum*, except during overnight fasting prior to neurobehavioral evaluations.
Water:	Tap water, *ad libitum*, except during overnight fasting prior to neurobehavioral evaluations.
Environmental conditions:	**Temperature:** 22±1EC **Humidity:** 40-70% **Air changes:** 12-15/hr **Photoperiod:** 12 hrs dark/ 12 hrs light
Acclimation period:	1 week

B. STUDY DESIGN

1. In-life dates: Start: May 9, 2005 End: May 27, 2005

2. Animal assignment and treatment: Animals were randomly assigned (stratified by body weight) to the test groups noted in Table 1. The animals were given a single gavage dose (10 mL/kg) of Mancozeb in 0.5% aqueous methylcellulose (Methocel™) then observed daily for 14 days. The animals were fasted overnight prior to dosing. Administration was staggered over a 4-day interval to facilitate neurobehavioral observations. At termination, a necropsy was performed on all animals.

TABLE 1. Study design [a]				
Experimental parameter	Dose (mg/kg)			
	0	500	1000	2000
Total number of animals/sex/group	10/sex	10/sex	10/sex	10/sex
Behavioral testing (FOB, Motor Activity)	10/sex	10/sex	10/sex	10/sex
Neuropathology	5/sex	5/sex	5/sex	5/sex

a Data were obtained from pages 17, 21, and 23 of the study report.

Dose rationale and time of peak effect: Previous toxicity information was provided from 4 studies: two acute oral toxicity studies in rats (Rohm and Hass report numbers 79R-180 and 83R 213B), a subchronic toxicity study in CD rats (MRID 00160704), and a subchronic neuropathology study in CD rats (MRID 42034101). The time-of-peak effect was determined in a probe study (study number not provided) using 5 Fisher 344 rats/sex/dose at 0 or 2000 mg/kg (limit dose). Based on the clinical signs observed (perineal fecal soiling and soft feces), the time of peak-effect was determined to be 5 hours post-dosing.

4. **Test Substance preparation and analysis:** Dose formulations were prepared daily prior to dosing by mixing the appropriate amount of Mancozeb (corrected for purity) with 0.5% aqueous methylcellulose. Homogeneity (top, middle, bottom) was verified for the 50 and 200 mg/mL formulations, and concentration analyses of all doses were performed on the first and last day of dosing. Prior to the start of the study, the stability of Mancozeb in the vehicle at room temperature was verified at all dose levels at 2, 6, and 24 hours.

Results

Homogeneity analysis (range as % relative standard deviation): 0.577-1.31%

Concentration analysis (range as mean % of nominal): 85.4-87.0%

Stability (range as % of initial after 24 hrs): 91.2-101%

The analytical data indicated that the mixing procedure was adequate and that the variation between nominal and actual dosage to the animals was acceptable.

5. **Statistics:** Statistical analyses were conducted on body weights (collected at FOB time points), grip performance, rectal temperature, landing foot splay, motor activity and FOB observations. The average of three grip performance trials and the average of three landing foot splay trials were statistically analyzed. Motor activity counts were reported as their square roots, reportedly "to minimize problems of heterogeneity of variance and departure from normality that commonly occur from treatment". For overall FOB summarization, ranked scores for each FOB observation (for males and females at each dose level) were converted into average scores for that observation. The average scores were descriptive only, and were not quantitatively analyzed.

FOB incidence scores were statistically analyzed by a z-test of proportions comparing each treated group to the control group. There were 16 mandatory graded observations, leading to a large number of z-tests which include the factors of sex, severity level, time point, and dose level comparisons.

Means and standard deviations were calculated for all continuous data, and homogeneity of variance was evaluated with the Bartlett's test ($\alpha = 0.01$). Body weights, rectal temperature, forelimb grip performance, hindlimb grip performance, landing foot splay and motor activity were analyzed by a factorial repeated-measures design, the multivariate approach, with factors of sex and treatment and the repeated factor of time. Motor activity also had the repeated factor of epoch (within time) in the model. The inclusion of pre-exposure data in the analysis made relevant only the analyses that included factors of both treatment and time since the treatment-by-time interaction assessed the true effect of treatment. The primary interactions examined at $\alpha = 0.05$ were:

Treatment × Time – A significant p value indicates that, taken together, both males and females were affected by treatment at some time point.

Treatment × Time × Sex – A significant p value indicates that treatment effects were different between males and females at some time point.

Treatment × Time × Epoch (motor activity only) – A significant p value indicates that the within-session distribution of motor activity counts was affected by treatment at some time.

The statistical significance of the treatment-by-time-by-sex interaction was examined first. If significant, the analysis was repeated separately for each sex. The treatment-by-time interaction and the treatment-by-time-by-epoch interaction were examined next. If either was significant, linear contrasts were calculated to determine which treatment groups were different from the control group. The comparison-wise error rate was set to $\alpha = 0.02$ to correct for multiple comparisons to the control. The Pillai Trace statistic was used to evaluate statistical significance. In the case of statistically significant linear contrasts, subsequent analyses may have been conducted to identify which time interval was different.
Assuming that the continuous data were normally distributed, the statistical methods were considered appropriate.

C. METHODS / OBSERVATIONS

1. **Mortality and clinical observations:** Animals were observed at least once daily for clinical signs of toxicity and at least twice daily for morbidity, mortality, and availability of food and water. Detailed physical examinations outside the home-cage were performed on Days 2-4.

2. **Ophthalmoscopic examination:** The eyes of all animals were examined prior to exposure.

3. **Body weight:** Animals were weighed as part of the FOB, during pre-exposure, on the day of dosing (Day 1), and on Days 8 and 15. An additional measurement was taken on Day 2 to aid in characterization of acute effects. Day 2 body weights were on non-fasted animals and

were not included in the statistical analyses.

4. **Food consumption:** Food consumption was not recorded.

5. **Cholinesterase determination:** Cholinesterase activity was not evaluated.

6. **Neurobehavioral assessment**

a. **Functional Observational Battery (FOB):** All animals were subjected to a FOB during pre-exposure (baseline), at Day 1 (5 hours post-dosing, time-of-peak effect), and Days 8 and 15. The FOB was conducted on rats randomly selected and presented to an observer who was 'blind' to the treatment status of the animal. All FOB testing was performed under red light conditions at approximately the same time each test day, and the same observer performed all of the evaluations. The FOB included: cage-side, hand-held and open-field observations and measurements of body weight, rectal temperature, fore- and hindlimb grip performance, and landing foot splay. The scoring criteria for the FOB were provided on pages 311-314 of the study report. The time in the open-field was at least 1 minute. The following CHECKED (X) parameters were examined.

	HOME CAGE OBSERVATIONS		HANDLING OBSERVATIONS		OPEN FIELD OBSERVATIONS
X	Posture*	X	Reactivity*	X	Mobility
X	Biting	X	Ease of removal	X	Rearing+
X	Convulsions*	X	Lacrimation*/chromodacryorrhea	X	Arousal/ general activity level*
X	Tremors*	X	Salivation*	X	Convulsions*
X	Abnormal movements*	X	Piloerection*	X	Tremors*
X	Palpebral closure*	X	Fur appearance	X	Abnormal movements*
X	Feces consistency	X	Palpebral closure*	X	Urination / defecation*
		X	Respiratory rate+	X	Grooming
	SENSORY OBSERVATIONS	X	Red/crusty deposits*	X	Gait abnormalities / posture*
	Approach response+	X	Mucous membranes /eye /skin color	X	Gait score*
X	Touch response+	X	Eye prominence*	X	Bizarre / stereotypic behavior*
X	Startle response*	X	Muscle tone*	X	Backing
X	Pain response*	X	Pupil size		Time to first step
X	Pupil response*		**PHYSIOLOGICAL OBSER.**		
	Eye blink response	X	Body weight*		**NEUROMUSCULAR OBSER.**
	Forelimb extension	X	Body temperature+		Hindlimb extensor strength
	Hindlimb extension		**OTHER OBSERVATIONS**	X	Forelimb grip strength*
	Air righting reflex+	X	Extensor thrust response	X	Hindlimb grip strength*
	Olfactory orientation			X	Landing foot splay*
					Rotarod performance

* Required parameters; + Recommended parameters

Fore- and hindlimb grip strength were measured (g) using a Chatillon electronic strain gauge (Greensboro, NC); the average value from 3 trials was used for statistical analysis. Landing foot splay was measured by applying ink to the outermost toes on the hind feet of the animal and dropping the animal from a height of 30 cm. The distance from center-to-center of the ink marks, for each trial, was measured (cm) and the average of the 3 splay values was used for statistical analysis. Rectal temperature was measured by carefully placing a Physitemp rectal thermistor (Clifton, NJ) approximately 4 cm into the rectum for approximately 10 seconds. Temperature was then recorded. The thermistor was validated at 37°C before and after the study.

b. **Locomotor activity:** Locomotor activity was evaluated pre-exposure (baseline), at Day 1 (5 hours post-dosing, time-of-peak effect), and Days 8 and 15. An automated system was used for motor activity data collection. No entry into the test room was allowed during the testing period. Each test session consisted of six 8-minute epochs, totaling 48 minutes of testing per animal per test session. Activity counts for each epoch were recorded. Rats were allocated to the motor activity cages in such a way that the counterbalancing of treatment groups and sexes across cages and test times was maximized.

7. **Sacrifice and pathology:** The animals selected for evaluation of neuropathological effects (5 rats/sex/dose) were given an intraperitoneal injection of 0.2 mL heparin (10,000 USP/mL) per 100 grams body weight approximately 10 minutes prior to perfusion. The animals were anesthetized (isoflurane) and perfused with 0.05M phosphate buffer containing sodium nitrite followed by a phosphate-buffered solution of 1.5% glutaraldehyde - 4% formaldehyde. The remaining 5 rats/sex/dose were euthanized by decapitation following CO_2 anesthesia and a standard set of tissues were saved in neutral, phosphate-buffered 10% formalin.

   Tissues were examined for gross pathologic alterations by a veterinary pathologist. The brain, head, spinal column with spinal cord, fore- and hindlimbs, and tail were trimmed to remove excessive skin and muscle; muscles from the hindlimbs were reflected to further expose the nerves. All tissues were immersed in glutaraldehyde/formaldehyde fixative. In addition, thoracic and abdominal viscera were collected and preserved in glutaraldehyde/ formaldehyde fixative. The following CHECKED (X) tissues were collected.

	CENTRAL NERVOUS SYSTEM		PERIPHERAL NERVOUS SYSTEM
	BRAIN		**SCIATIC NERVE**
X	Olfactory bulb	X	Mid-thigh
X	Cerebrum (frontal, parietal, temporal, and occipital lobes)		Sciatic notch
X	Thalamus/hypothalamus		
X	Midbrain		
X	Pons		**OTHER**
X	Cerebellum	X	Sural nerve
X	Medulla oblongata	X	Tibial nerve (proximal and distal)
	SPINAL CORD		Peroneal nerve
X	Cervical swelling	X	Lumbar dorsal root ganglion
X	Lumbar swelling	X	Lumbar dorsal root fibers
	Thoracic swelling	X	Lumbar ventral root fibers
	OTHER	X	Cervical dorsal root ganglion
	Gasserian ganglion	X	Cervical dorsal root fibers
X	Trigeminal ganglion and nerve	X	Cervical ventral root fibers
X	Pituitary gland		
X	Eyes w/optic nerve		
X	Olfactory epithelium		
X	Gastrocnemius muscle		
X	Anterior tibial muscle		

The collected tissues from all perfused animals in the control and 2000 mg/kg group were further processed for microscopic evaluation. Nine cross-sections of the brain were prepared from the following structures: olfactory bulb, cerebrum (frontal, parietal, temporal and occipital lobes), thalamus/hypothalamus, midbrain, pons, cerebellum, and medulla oblongata. In addition, sections were prepared from the trigeminal ganglion and nerve, pituitary gland, eyes with optic nerves, spinal cord (cervical and lumbar), olfactory epithelium, and skeletal muscles (gastrocnemius and anterior tibial). These tissues were processed by standard histologic procedures, embedded in paraffin, sectioned approximately 6-µm thick and stained with hematoxylin and eosin.

Spinal nerve roots (cervical and lumbar), dorsal root ganglia (cervical and lumbar), and peripheral nerves (sciatic, tibial (proximal and distal - at the knee and calf muscle branches) and sural) were osmicated, embedded in epoxy resin, sectioned approximately 2 to 3 µm thick and stained with toluidine blue.

8. **Positive controls:** Summary data were provided from three studies that validate the motor activity test system (Dow Study ID #: 001189, Feb. 13, 2001), the laboratory's ability to identify neuropathological lesions (Dow Study ID #: T2.08-001-012-001), and the technician performing the FOB evaluations (Dow Study Report #: T1.05-022-000-014). In the motor activity study, rats received single i.p. injections of *d*-amphetamine (0.25 or 1 mg/kg), chlorpromazine (2 or 5 mg/kg), or saline immediately before initiation of motor activity evaluations. The test system detected the significant (p#0.05) increases (*d*-amphetamine) and decreases (chlorpromazine) in both total and within-session activity in the treated groups

compared to controls. The ability of the test system to detect habituation was also demonstrated. In the neuropathology study, rats were treated with either trimethyltin (7 mg/kg, single gavage), acrylamide (35 mg/kg/day, gavage for 5 days/week for 3 weeks), or distilled water (gavage for 5 days/week for 3 weeks). In the acrylamide group, very slight to slight axonal degeneration in the tibial, sural, and peroneal nerves was observed. Lesions attributed to trimethyltin included degenerative neuronal lesions in the hippocampus and piriform cortex of the central nervous system, and nerve fiber degeneration in the cervical and lumbar spinal cord sections, peroneal nerve, and proximal sciatic nerve. The technician's ability to properly identify FOB endpoints was demonstrated using rats treated via i.p. injection with saline (0.15 mL), *d*-amphetamine sulfate (8 mg/kg), chlorpromazine (4 mg/kg), or atropine (2 mg/kg) followed 5 minutes later with physiostigmine sulfate (0.75 mg/kg). The observer was 'blind' as to the animal treatment group.

II. RESULTS

A. OBSERVATIONS

1. **Clinical signs**: The only clinical signs noted were perineal fecal staining in 0/10, 1/10, 2/10, 1/10 males and 0/10, 2/10, 4/10, 3/10 females in the respective dose groups. The study author suggested that this may have occurred because mancozeb has antifungal properties and possible antibacterial properties which may have transiently affected the normal flora of the digestive tract.

2. **Mortality**: All animals survived to scheduled sacrifice.

3. **Ophthalmoscopic examinations**: There were no preexisting ophthalmoscopic conditions present in any animal.

B. BODY WEIGHT AND BODY WEIGHT GAIN: No biologically significant effects on body weight or body weight gain were observed at any dose (Table 2). It was stated that statistically significant decreases in body weight were observed in the 1000 and 2000 mg/kg groups; however, as these decreases were minor (↓2-3%), transient (the magnitude decreased from Days 8 to 15), and not dose-dependent in the females, these findings were not considered to be biologically significant. Similarly at 1000 mg/kg and above, minor decreases in overall (Days 1-15) body weight gains (calculated by the reviewers) were noted in the males (↓5-8%) and females (↓4-11%; not dose dependent). It should be noted that statistical significance was not denoted in the data tables.

TABLE 2. Mean (±SD) body weight and body weight gain (g) in rats exposed to Mancozeb once via gavage. [a]

Days	Dose (mg/kg)			
	0	500	1000	2000
Males				
1	86.1±4.5	87.2±8.0	87.6±6.8	87.0±6.6
2	99.8±4.0	100.3±8.9	99.9±7.2	98.8±6.4
8	109.4±3.8	109.0±12.3	107.2±8.5	105.9±8.8
15	132.2±4.2	133.1±15.9	131.5±10.5	129.6±12.0
Overall (1-15) weight gain [b]	46.1	45.9	43.9 (↓5)	42.6 (↓8)
Females				
1	80.3±4.0	79.0±3.9	80.3±5.1	80.2±5.7
2	92.8±3.8	90.3±3.7	90.9±6.5	91.7±5.5
8	98.7±3.5	95.4±5.1	95.3±6.6	95.7±6.8
15	112.5±4.9	109.0±5.5	108.8±4.1	111.0±5.7
Overall (1-15) weight gain [b]	32.2	30.0	28.5 (↓11)	30.8 (↓4)

a Data were obtained from Tables 11 and 12 on pages 61-62 of the study report; n=10. Percent difference from controls (calculated by reviewers) is presented parenthetically.

b Calculated by reviewers from data contained within this table.

C. FOOD CONSUMPTION: Food consumption was not reported.

D. CHOLINESTERASE ACTIVITIES: Cholinesterase activity was not evaluated.

E. NEUROBEHAVIORAL RESULTS

1. **FOB findings:** No treatment-related FOB effects were noted at any dose at any time point in either sex. There was a slight decrease in the level of activity in the open-field in the 2000 mg/kg males on Day 15. However, as this finding did not occur in a dose-related manner, was not statistically significant, and did not correlate with motor activity data on Day 15, it was considered incidental.

   Motor activity: The study report transformed the motor activity results by reporting square roots of the motor activity data. The untransformed data were also reported, but means and standard deviations were not reported for the untransformed data. The study report explained using the square roots of the data with this statement:

   > "Motor activity counts were reported as their square roots to minimize problems of heterogeneity of variance and departure from normality that commonly occur from treatment (Pryor *et al.*, 1983)."

   Reporting motor activity results as square roots is very unusual and minimized the changes which occurred. For example, using the square root data showed only a 14% decrease in activity for low-dose males on day 1, but the actual results were a 25% decrease in activity using the untransformed data. Because reporting the square root of the data minimizes the changes which occurred to treatment, this review reports only the untransformed data which are shown in Table 3 below. Statistical significance was not reported in the data tables. The interval (epoch) data are attached in Tables 4 and 5 in the Appendix.

The motor activity data using untransformed results are shown below in Table 3. On day 1 there was decreased total session motor activity in comparison to controls in all male groups (-25% to -31%) and all female treatment groups (-20% to -35%). The dose-response curve for day 1 was very flat for the 3 treatment groups. The baseline motor activity was very comparable between all groups for both sexes. The study report stated that the motor activity was within the historical control ranges.

TABLE 3. Mean (±SD) total session motor activity) in rats exposed to Mancozeb once via gavage.

Test day	0 mg/kg	500 mg/kg	1000 mg/kg	2000 mg/kg
Males				
Baseline	108.9 ± 58.6	101.1 ± 28.6 (-7%)	112.1 ± 37.7 (+3%)	110.1 ± 68.8 (+1%)
Day 1	117.5 ± 45.6	87.9 ± 34.0 (-25%)	80.7 ± 21.6 (-31%)	87.9 ± 32.4 (-25%)
Day 8	136.0 ± 67.7	152.1 ± 45.6 (+12%)	129.7 ± 44.9 (-5%)	159.2 ± 38.6 (+17%)
Day 15	153.1 ± 32.0	174.2 ± 36.9 (+14%)	167.2 ± 44.7 (+9%)	159.3 ± 43.7 (+4%)
Females				
Baseline	107.2 ± 49.9	110.3 ± 61.6 (+3%)	118.0 ± 48.6 (+10%)	115.0 ± 51.8 (+7%)
Day 1	144.6 ± 33.9	116.0 ± 31.7 (-20%)	93.3 ± 30.4 (-35%)	97.4 ± 29.3 (-33%)
Day 8	136.5 ± 40.8	170.5 ± 32.0 (+25%)	172.6 ± 31.5 (+26%)	145.3 ± 60.1 (+6%)
Day 15	175.5 ± 28.3	179.8 ± 33.7 (+2%)	168.3 ± 30.7 (-4%)	156.5 ± 27.8 (-11%)

Data from Appendix Table 19, pages 198-205 of the study report, untransformed motor counts for intervals 1-6. Mean and s.d. calculated by reviewer. Number in parentheses = % of control, calculated by reviewer. n=10
NOTE: Statistical significance not calculated.

F. SACRIFICE AND PATHOLOGY

1. **Gross pathology:** No treatment-related gross lesions were observed at any dose.

2. **Brain weight:** Brain weights were not reported.

3. **Neuropathology**: Degeneration of an individual nerve fiber with myelin ovoid formation was seen in the proximal sciatic nerve of one male in the 2000 mg/kg group and in the tibial nerve of two males in this dose group. Although affecting only one nerve in these 3 animals, these lesions were similar to those seen in the subchronic neuropathology study with mancozeb and are attributed to treatment.

III. DISCUSSION AND CONCLUSIONS

A. INVESTIGATOR'S CONCLUSIONS:

The Sponsor concluded that a single oral gavage dose of Mancozeb at ≥1000 mg/kg induced: (i) perineal fecal soiling in a small number of rats (1-4/dose); (ii) transient decreases in body weight in both sexes; and (iii) a transient decrease in total session motor activity on Day 1 that was associated with potential systemic toxicity rather than a neurotoxic effect. The NOAEL for systemic effects is 500 mg/kg. The NOAEL for neurotoxicity is 2000 mg/kg.

B. REVIEWER COMMENTS:

Decreases in total session motor activity occurred on Day 1 in all 3 male and female treatment groups. The fact that the decreased motor activity was seen on the day of treatment is significant. The decrease in motor activity did not show a dose-related response, however there was histopathology of the nervous system at the high dose.

Degeneration of individual nerve fibers with myelin ovoid formation was seen in 3 high-dose males in this acute study. Although affecting only one nerve in each animal, these lesions were similar to those seen in the subchronic neuropathology study with mancozeb and are therefore attributed to treatment. In the subchronic neuropathology study, there was myelin damage to sciatic and tibial nerves which included myelin ovoid formation at dietary doses equivalent to 50 mg/kg/day and above. Males were more sensitive than females regarding myelinopathy in the subchronic study as they were in this acute study.

The study author attributed the decreased motor activity to systemic toxicity which included increased perineal fecal soiling, decreased body weight (-2% to -3% compared to controls), and decreased rectal temperature (-1% compared to controls). However, these changes were very minor and not considered toxicologically significant.

The LOAEL is 500 mg/kg/day, the lowest dose tested, based on decreased motor activity on day 1 in males and females. The NOAEL was not determined.

C. STUDY DEFICIENCIES: The following deficiencies were noted, but do not change the conclusions of this DER:

- Statistical significance was not shown in the data tables.
- As noted above, results for motor activity emphasized square roots of the actual data rather than analyzing the actual untransformed data.
- Brain weights were not reported.

Appendix

Table 4. Male Motor Activity, Day 1							
Animal	**Epoch 1**	**Epoch 2**	**Epoch 3**	**Epoch 4**	**Epoch 5**	**Epoch 6**	**Total**
Controls							
3813	43	33	0	0	0	2	78
3814	50	32	31	15	20	21	169
3815	47	17	23	14	13	0	114
3816	29	28	26	27	17	10	137
3817	37	16	0	0	0	0	53
3818	42	29	15	27	35	32	180
3819	46	28	13	0	0	0	87
3820	49	45	29	24	17	10	174
3821	29	22	12	12	0	0	75
3822	57	20	20	11	0	0	108
						mean	**117.5**
						S.D.	45.6
500 mg							
3823	47	29	22	0	0	7	105
3824	34	12	0	0	0	0	46
3825	42	25	7	9	0	1	84
3826	42	17	9	0	0	0	68
3827	43	35	1	0	0	5	84
3828	21	3	11	0	0	0	35
3829	48	26	14	20	14	0	122
3830	67	28	8	1	29	0	133
3831	37	45	32	4	0	11	129
3832	44	20	9	0	0	0	73
						mean	**87.9**
						S.D.	34.0
1000 mg							
3833	24	1	0	3	5	0	33
3834	37	13	0	0	13	0	63
3835	42	16	17	12	0	0	87
3836	67	31	7	0	0	0	105
3837	46	31	7	0	0	0	84
3838	39	24	11	0	0	10	84
3839	55	24	15	0	1	0	95
3840	39	26	0	0	0	0	65
3841	53	16	0	0	15	5	89
3842	59	31	12	0	0	0	102
						mean	**80.7**
						S.D.	21.6

Table 4. Male Motor Activity, Day 1

Animal	Epoch 1	Epoch 2	Epoch 3	Epoch 4	Epoch 5	Epoch 6	Total
2000 mg							
3843	27	17	0	7	5	0	56
3844	56	8	0	0	0	10	74
3845	41	23	14	0	5	0	83
3846	41	8	14	0	0	0	63
3847	33	26	9	1	9	1	79
3848	48	26	13	0	11	0	98
3849	31	21	35	16	22	36	161
3850	39	29	30	16	0	0	114
3851	35	30	0	0	5	29	99
3852	29	2	0	18	3	0	52
						mean	**87.9**
						S.D.	32.4

Data from Appendix Table 19, pages 198-204 of the study report. Mean and s.d. calculated by reviewer.

Table 5. Female Motor Activity, Day 1

Animal	Epoch 1	Epoch 2	Epoch 3	Epoch 4	Epoch 5	Epoch 6	Total
Controls							
3853	49	19	16	23	30	26	163
3854	31	8	27	5	0	6	77
3855	23	13	18	12	15	19	100
3856	39	33	18	25	23	19	157
3857	42	45	20	14	27	3	151
3858	44	31	22	19	27	16	159
3859	53	30	26	34	25	30	198
3860	29	28	17	25	7	42	148
3861	32	32	22	14	21	17	138
3862	52	42	47	6	8	0	155
						mean	**144.6**
						S.D.	33.9
500 mg							
3863	36	23	7	20	5	0	91
3864	36	25	30	18	5	0	114
3865	64	22	3	16	31	0	136
3866	24	29	19	23	6	0	101
3867	38	24	0	0	9	14	85
3868	54	70	20	6	13	0	163
3869	44	18	14	4	0	0	80
3870	40	46	21	27	9	14	157
3871	49	38	1	0	0	0	88
3872	28	22	28	15	22	30	145
						mean	**116**
						S.D.	31.7

Table 5. Female Motor Activity, Day 1

Animal	Epoch 1	Epoch 2	Epoch 3	Epoch 4	Epoch 5	Epoch 6	Total
1000 mg							
3873	48	42	19	4	4	3	120
3874	37	25	27	20	14	10	133
3875	31	25	19	12	0	0	87
3876	30	12	0	0	0	0	42
3877	36	23	7	1	0	0	67
3878	65	25	13	4	6	0	113
3879	47	20	0	0	0	33	100
3880	25	9	19	0	0	2	55
3881	45	30	14	26	0	6	121
3882	40	26	0	2	0	27	95
						mean	**93.3**
						S.D.	30.4
2000 mg							
3883	61	6	14	0	15	0	96
3884	46	48	8	24	0	11	137
3885	60	16	0	0	7	3	86
3886	43	23	0	0	0	5	71
3887	41	10	7	0	0	0	58
3888	49	8	27	17	19	0	120
3889	32	21	0	19	0	0	72
3890	69	27	14	0	0	38	148
3891	31	24	31	3	0	0	89
3892	58	17	0	0	6	16	97
						mean	**97.4**
						S.D.	29.3

Data from Appendix Table 19, pages 199-205 of the study report.
Mean and s.d. calculated by reviewer.